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ORIGINAL ARTICLE

Coagulation profiles during and after anabolic androgenic steroid use: data from the HAARLEM study

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Abstract

Background: Anabolic androgenic steroids (AAS) are thought to increase venous thromboembolism (VTE) risk.

Objectives: We investigated whether AAS influence coagulation parameters associated with VTE by assessing their changes during and after AAS use.

Methods: The HAARLEM study enrolled 100 male amateur athletes voluntarily starting an AAS cycle between 2015 and 2018. We measured procoagulant and anticoagulant protein levels, D-dimer levels, endogenous thrombin potential (ETP), and clot lysis time (CLT) at baseline and during 2 years of follow-up. Changes in coagulation during AAS cycle, 3 months after its discontinuation, and 1 year after its inclusion compared with baseline were estimated using linear mixed models. The associations between AAS dose and duration of use with these outcomes were studied through adjusted multivariable linear regression.

Results: Participants used AAS for a median of 13 weeks (IQR: 10-23) with a median weekly dose of 901 mg (IQR: 634-1345 mg). Mean levels of multiple coagulation factors (F) increased during use compared with baseline, whereas FVIII and von Willebrand factor levels remained unchanged. Protein S and D-dimer showed the biggest increase (22% [95% CI: 15-29] and 1.3-fold [95% CI: 1.2-1.5], respectively). CLT was 8 minutes longer (95% CI: 5-10) and ETP was 165 nM*min (95% CI: −205 to −124) lower during the AAS cycle. A high weekly AAS dose and short cycle duration were associated with changes in protein S and ETP during use. All parameters returned to baseline values 3 months after discontinuation and remained similar after.

Conclusion: During AAS use, procoagulant and anticoagulant protein levels increased in a reversible manner. The overall balance did not suggest a clear procoagulant state.

KEYWORDS
anabolic androgenic steroids, blood coagulation, fibrin clot lysis time, testosterone, venous thromboembolism

Essentials

- Anabolic androgenic steroids (AAS) might increase venous thromboembolism risk.
- We investigated the effect of AAS on coagulation and fibrinolysis in amateur strength athletes.
- Procoagulant and anticoagulant factors reversibly increased during the AAS cycle and thrombin generation decreased.
- The observed changes do not point toward a clear procoagulant state during steroid use.

1 | INTRODUCTION

Anabolic androgenic steroids (AAS) are synthetic derivates of testosterone, the male sex hormone. These substances belong to the class of performance and image-enhancing drugs (PIEDs), commonly used to gain muscle mass and strength and to increase the oxygen carrying capacity of blood [1,2]. Although the word "doping" is traditionally associated with professional athletes, the majority of AAS users are amateur bodybuilders or frequenters of gyms. It is estimated that approximately 1% to 6% of regular visitors of gyms use AAS [3,4]. However, in 2009, the Netherlands Organization for Applied Scientific Research (TNO) found an even higher prevalence (>8%) among visitors of fitness centers [5]. As in most countries, the production and trading of AAS without a license is illegal in the Netherlands; however, AAS can be easily acquired through local dealers or the internet [6].

AAS use is generally regarded as harmful. Long-term use of AAS is associated with increased risk of arterial cardiovascular events [7,8]. Increased risk of venous thromboembolism (VTE) has also been linked to AAS [9,10], but the level of evidence is low, primarily based on case reports and few small epidemiologic studies [11]. In addition, data are lacking on the underlying mechanism. AAS are thought to potentiate both activation and regulation of coagulation, which may translate into an increased VTE risk when using a longer AAS cycle. The effect of AAS on coagulation has been studied by employing a single type of androgen in intervention studies as therapy for coagulation factor (F) deficiencies, or in small cross-sectional studies of AAS abusers [11]. Several studies showed that AAS use is associated with increased levels of FII, FIX, and FVIII [12,13], yet not all studies confirmed these findings [12,14,15]. Levels of coagulation inhibitors, such as protein S (PS), were also found to be increased [12,14]. Limited data are available on thrombin generation parameters, as only one study showed an augmented coagulation potential in AAS users [12]. Nevertheless, some data are case reports and most studies lack power due to a modest number of subjects included. Moreover, only short-term intervention studies have addressed the effect of AAS on hemostasis, while long-term effects or the extent of recovery after AAS withdrawal have not been studied.

Our aim was to investigate the effect of an AAS cycle on the coagulation system in a population of amateur strength athletes. Moreover, we aimed to assess whether these changes are influenced by androgen dose, duration of use, and route of administration and to investigate the extent of eventual recovery after AAS withdrawal.

2 | METHODS

2.1 | Study design and subjects

This study used data from the HAARLEM study (Health risks of Anabolic Androgenic steRoid use by maLE aMateur athletes), details of which have been previously described [16]. Briefly, the HAARLEM study is a cohort study that enrolled 100 men aged ≥18 years intending to start a self-initiated cycle of AAS between October 2015 and May 2018. After ethical approval, the study was widely promoted on national television and in regional newspapers in order to reach as many potential participants as possible. Moreover, the study was also promoted on several websites (eg, bodybuilding discussion forums, news websites for health and fitness), which are known to be visited frequently by men that engage in strength sport. Subjects were included if they intended to start an AAS cycle within 2 weeks after signing informed consent. The scheduled AAS cycles were to be of at least 6 weeks duration and comprise at least 2 different types of AAS with a weekly dose of at least 200 mg. The AAS had to be acquired through participants' usual channels and were not provided or reimbursed by the investigators. Subjects did not receive any reward or reimbursement for participating in the study. The subjects were enrolled within 14 days before the beginning of a new cycle (T_0) and then followed-up at fixed time points (for a total of 4 additional study visits, up to 2 years after inclusion, so that the last plasma sample was collected in March 2020). The study design required the subjects to completely discontinue AAS use after the cycle within the first 1 year of follow-up in order to assess adverse events after androgen exposure and potential recovery. Between 1 and 2 years of follow-up, subjects were allowed to use AAS in the way they chose. Exclusion criteria were the use of AAS and the diagnosis of a new chronic somatic or psychiatric illness within 3 and 6 months prior to inclusion, respectively. Men were also excluded from participation if, within 6 months prior inclusion, treatment for a chronic illness had been started or altered significantly or a somatic illness (other than trauma) or a psychiatric illness had led to hospital or psychiatric institution admission. For the current analysis, we additionally excluded subjects treated with anticoagulants during the study period.

The AAS cycle performed by the participant was recorded meticulously, including the duration and dosage of each type of AAS. In addition, the use of drugs of abuse, other PIEDs (eg, growth hormone, thyroid hormone, and clenbuterol), medications (selective

estrogenic receptor modulators and aromatase inhibitors), and post-cycle therapy (PCT), used to prevent testosterone withdrawal symptoms after the cycle, was documented. As the use of other PIEDs [17], PCT, and other recreational drugs [18,19] may also influence coagulation, their use was accounted for in the statistical analysis.

The study was approved by the local Medical Ethical Committee (MEC registration: M015-019; study number: NH015.189) in October 2015.

2.2 | Outcome: measures of coagulation

After enrollment, subjects visited the laboratory for baseline blood analysis (T_0). Clinic visits with repeated blood analysis were followed at 4 additional fixed time points: 1) during steroid use (in the last week of the AAS cycle, T_1), 2) 3 months after discontinuing AAS (T_2), 3) 1 year after inclusion (T_3), and 4) 2 years after inclusion (T_4). Plasma levels of coagulation factors (FII, FVIII, FIX, and FXI), free PS, von Willebrand factor antigen (VWF), and D-dimer levels were measured at each time point. All coagulation-related laboratory measurements were analyzed on an ACL-Top 700 analyzer (Instrumentation Laboratory). FVIII, FIX, and FXI levels were measured using modified activated partial thromboplastin time assays and FII level was measured using a modified prothrombin time assay using immunodepleted plasmas. VWF, D-dimer, and free PS levels were measured using an automated latex enhanced immunoassay using the HemosIL VWF:Ag, HemosIL D-dimer HS 500, and HemosIL Free Protein S reagent kit, respectively. Lysis of a tissue factor–induced clot by exogenous tissue-type plasminogen activator (t-PA) was studied by monitoring changes in turbidity during clot formation and subsequent lysis (clot formation time and clot lysis time [CFT and CLT, respectively]), as previously described [20]. The generation of thrombin in clotting plasma was analyzed by means of a thrombomodulin-modified thrombin generation assay (TGA) via the calibrated automated thrombogram assays using a microtiter plate reading fluorometer (Fluoroskan Ascent, ThermoLab Systems) and Thrombinoscope software (Thrombinoscope BV) according to the manufacturer's specifications [21]. We determined the lag time of the thrombin formation process (in minutes), the peak thrombin concentration (peak, nM), the effective rate of thrombin generation between lag time and time to peak (velocity index, nM/min), and the endogenous thrombin potential (ETP, nM*min) recording the total amount of thrombin formed. CFT, CLT, and TGA parameters were measured only up until T_3 due to logistic reasons.

2.3 | Statistical analysis

Mean levels and SDs of all parameters were calculated at baseline (T_0) and at each follow-up visit (T_1, T_2, T_3, and T_4). Coagulation parameters were visually checked for a normal distribution, and D-dimer was log-transformed to achieve an approximately normal distribution. A linear mixed model, with unstructured covariance matrix using restricted maximum likelihood, was used to assess changes of coagulation measurements during follow-up up until T_3. Estimates and 95% CIs on the log scale were back-transformed to the original scale. To investigate differences by route of AAS administration, we further stratified the analysis between subjects using only injectable steroids and subjects who combined oral and injectable steroids at the time of blood draw at T_1.

To assess the measurement at 2 years of follow-up (T_4), we stratified the subjects in 3 groups according to their AAS use between 1 and 2 years of follow-up: subjects who did not perform another AAS cycle (non-users); subjects who performed ≥1 cycles, but were not taking AAS at the time of sampling (repeat cycle users); and subjects who were using AAS at the time of blood draw (current users). We compared coagulation parameters of non-users and cycle users with their baseline measurement (T_0) to assess the extent of their recovery. Instead, measurements of current users were compared to their T_1 measurement (during their first steroid cycle) to investigate the possibility of additional changes due to repeated and prolonged use.

We used multivariable linear regression to assess the association between weekly cycle dose and duration of use with changes in coagulation parameters between baseline and during AAS use ($\Delta T_1 = T_1 - T_0$). In a second model, we further adjusted for possible confounders (the number of different AAS used, the use of AAS at time of T_1, the use of other PIEDs, and recreational drug use).

To assess the association between cycle dose and duration with recovery, we included in a third model as outcome variable the difference between the coagulation measurements at baseline and at 3 months after withdrawal of AAS ($\Delta T_2 = T_2 - T_0$) with cycle dose or duration as exposure. In the fully adjusted model, the use of postcycle therapy was also included, together with the previously mentioned variables. As a sensitivity analysis, to ensure that recreational drugs did not influence our results, we performed the aforementioned analyses in a subgroup of nondrug users.

All analyses were performed with SPPS Statistics (25.0) and R Studio, R version 3.4.4. (R Foundation of Statistical Computing).

3 | RESULTS

3.1 | Study population

A total of 100 men were enrolled in the HAARLEM study (Table 1); of them, 99 were eligible for analysis as 1 subject was excluded due to anticoagulant use. Their median age was 30 years (IQR: 26-37 years). The median androgen dose of the AAS cycle performed during follow-up was 901 mg/wk (IQR: 634-1345), with a median of 4 different types of AAS (IQR: 3-5). The median cycle duration was 13 weeks (IQR: 10-23 weeks). Seventy-six patients followed the study protocol until T_3, as 2 subjects withdrew consent, 5 continued AAS use after T_1, 10 started a new cycle between T_2 and T_3, and 7 had missed visits. In these 24 subjects, T_2 and/or T_3 measurements were not used for analysis of recovery [22]. Between 1 and 2 years of follow-up, 23

TABLE 1 Characteristics of the HAARLEM population at baseline (n = 99).

General	
Age, y; median (IQR)	30 (26-37)
Men (%)	99 (100%)
Height (cm), mean (SD)	182 (7)
Weight (kg), mean (SD)	89 (13)
BMI (kg/m^2), mean (SD)	26 (3)
Previous AAS use (%)	79 (79%)
Recreational drug use (%)	62 (62%)
Current sport	
Fitness/bodybuilding (%)	92 (93%)
Competitive bodybuilding (%)	19 (19%)
Weight lifting (%)	3 (3%)
Strongman athlete (%)	3 (3%)
Combat sports (eg, kickboxing, karate, and judo), %	44 (44%)
Characteristics of AAS cycle	
Cycle duration (wk), median (IQR)	13 (10-23)
Types of AASs used, median (IQR)	4 (3-5)
Cumulative cycle dose (mg), median (IQR)	13100 (770-22825)
Average weekly dose (mg), median (IQR)	901 (634-1345)

(23%) subjects did not use AAS again, whereas 32 (32%) performed 1 or multiple new cycles, and 22 (22%) subjects used AAS throughout the second year.

3.2 | Changes in coagulation parameters between baseline and follow-up

Mean levels of the coagulation factors FII, FIX, and FXI increased during steroid use (T_1) compared to T_0 (Figures 1–3 and Supplementary Table S1), whereas FVIII levels remained unchanged and VWF levels decreased. Levels of the natural anticoagulant PS also increased, with a mean difference of 22% (95% CI: 15-29) between T_1 and T_0. D-dimer levels were 1.3 times higher during AAS than at baseline (95% CI: 1.2-1.5). CFT and CLT were prolonged at T_1 compared to T_0, whereas all thrombin generation parameters decreased at T_1, except for the lag time. ETP was 165 nM*min (95% CI: 124-205) lower at T_1 compared to baseline. Three months after AAS withdrawal (T_2), all coagulation parameters were restored to baseline value and did not change 1 year after enrollment (T_3). Two years after inclusion, mean levels of coagulation parameters in nonusers and repeat cycle users were similar to their baseline level (Supplementary Table S2). Current users (subjects using AAS at the 2-year visit [T_4]) had coagulation factor levels similar to their measurement during the first cycle.

3.3 | Differences in administration route

Subjects using oral AAS had higher mean levels of FII, FIX, and FXI and longer CFT and CLT at T_1 (Figure 3) than users of injectable AAS. No differences were observed for any of the parameters at the other time points (T_0, T_2, and T_3), except for FVIII and VWF mean levels that were lower at all time points (including baseline) in oral AAS users than those in parenteral AAS users (data not shown). As for thrombin generation parameters, ETP, peak, and velocity index were lower, whereas lag time was increased at T_1 in oral steroid users compared to injectable AAS users.

3.4 | Association between cycle characteristics and changes in coagulation parameters

In the crude model, higher weekly dose and shorter cycle duration were positively associated with the increase of FII, FIX, and PS between T_1 and T_0. In the adjusted model, only the increase in PS and changes in TGA were associated with a higher dose and shorter duration (Table 2). Neither weekly dose nor cycle length was associated with the recovery of coagulation parameters (T_2–T_0), except for TGA parameters, in both the unadjusted and adjusted models (Supplementary Table S3).

3.5 | Sensitivity analysis

In the 37 subjects who did not report recreational drug use, all parameters at all time points were similar to those of recreational drug users. The results of the linear mixed model in the subgroup of nondrug users were similar to the main analysis, with similar mean differences. Due to relatively small size of this subgroup, we did not have enough power to investigate the association between cycle dose and duration and changes in coagulation parameters.

4 | DISCUSSION

In this study, we investigated changes in coagulation parameters during and after AAS use in a cohort of amateur strength athletes. We observed that AAS use affected different stages of the activation and inhibition of the coagulation cascade as reflected by an increase of procoagulant and anticoagulant factors during AAS cycle. Of interest, we observed major differences in thrombin generation curves before and during AAS use. The time to initiation of coagulation was prolonged during androgen exposure and all other TGA parameters, in particular ETP, were decreased. Nonetheless, AAS use resulted in D-dimer increase, which suggests a minor *in vivo* activation of coagulation and fibrinolysis despite an *ex vivo* anticoagulant effect. Also, the observed increase in CLT suggests a procoagulant effect of AAS use. All parameters returned to baseline values 3 months after discontinuation and remained similar after.

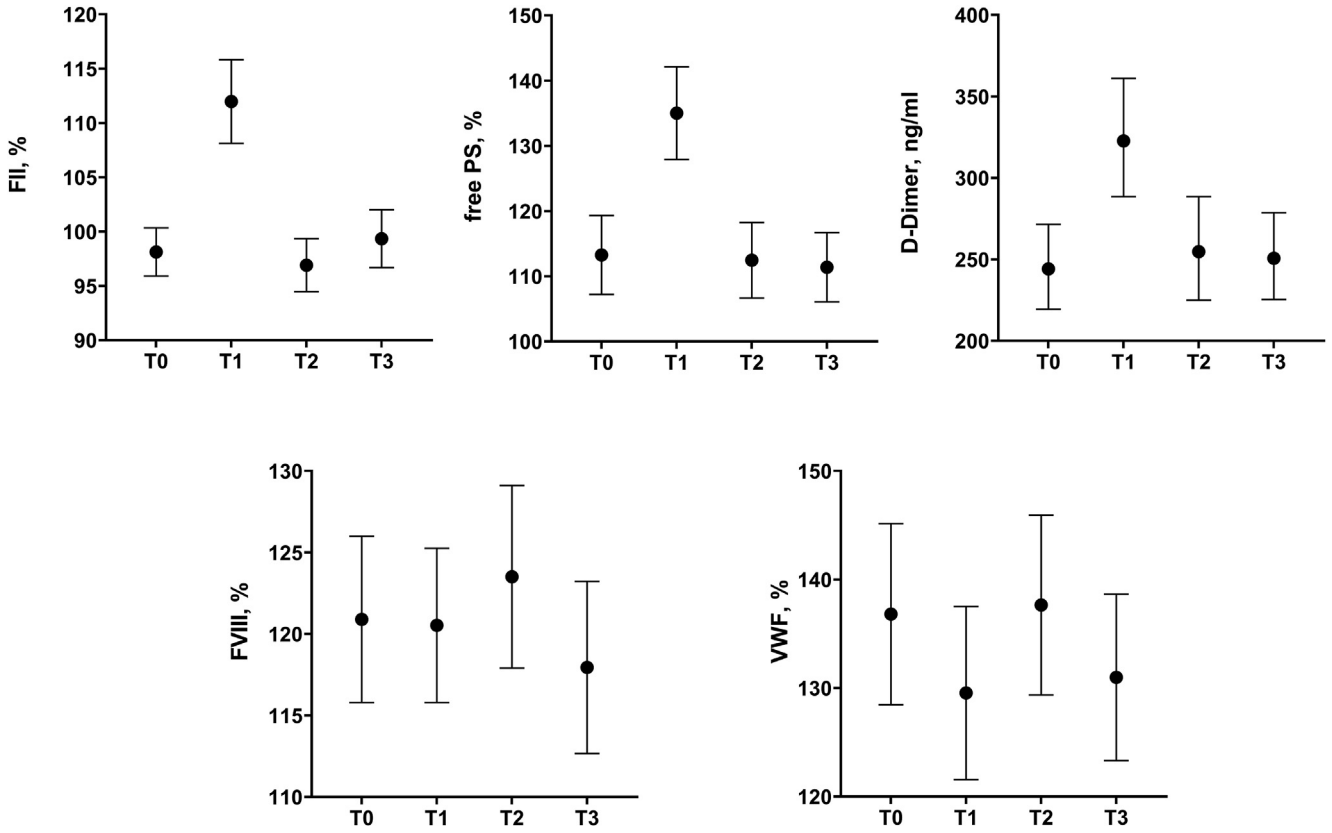

FIGURE 1 Estimated marginal means of coagulation parameters at baseline, during and after AAS use, obtained from a linear mixed model. Anchored lines indicate 95% CIs. F, factor; PS, protein S; VWF, von Willebrand factor.

Only few previous cross-sectional studies investigated the effect of AAS on coagulation in AAS users and reported an increase in several procoagulant and anticoagulant parameters [12,14,23] and fibrinolytic impairment [24]. Similarly, only 1 previous study evaluated TGA [12] and reported an increased ETP in AAS users in comparison with controls. On the contrary, we observed a decrease in ETP, which might be explained by the different TGA used. While Chang et al. used a standard TGA, we performed a thrombomodulin-modified TGA. In this latter assay, the capacity of the protein C system to downregulate coagulation is taken into account. As an increase of protein C during AAS use was previously reported [12,14,23], this might explain the reduced thrombin potential we observed. Furthermore, Chang et al. [12] also observed differences in coagulation parameters between former AAS abusers, who had stopped AAS, and controls. In contrast, all parameters were completely restored to baseline levels in our population, up to 1 year after AAS withdrawal. This remained consistent in the analysis at 2-year follow-up stratified by AAS use when participants could perform a second cycle if they wanted. Taken all together, our findings point toward the complete reversibility of the changes after AAS withdrawal. However, it cannot be entirely excluded that multiple years of consecutive AAS use may affect coagulation irreversibly.

Interestingly, as shown in Supplementary Figure S1 and by the increased residual covariance of the linear mixed model during AAS (Supplementary Table S4), the changes in coagulation parameters were heterogenous between subjects. This may be due to individual (ie, genetic) differences. Alternatively, different AAS types might have a different effect on coagulation, as suggested by previous studies [15,25]. Our study population used diverse combinations of AAS in different durations and dosages. In our results, cycle dose and duration of use were associated only with an increase in PS during AAS, and a decrease and subsequent recovery of TGA parameters. Nevertheless, we used information from the label to estimate the dose of the AAS compounds. This only serves as a rough estimate and may have clouded the "real" dose effect. Furthermore, as AAS types could not be reliably derived from label information, we could not assess the effect of specific compounds. Finally, the different routes of AAS administration could also partly explain between-subject differences, as we observed the greatest changes in oral AAS users. Oral compounds might cause higher peak plasma concentrations or have an immediate effect on the liver due to first-pass metabolism [26,27].

As we observed changes in both procoagulant and anticoagulant parameters, it is difficult to estimate whether an overall procoagulant or anticoagulant shift in the balance takes place. The reduced TG potential would suggest that the anticoagulant changes prevail over the procoagulant ones. Moreover, FVIII and VWF, the hemostatic factors which are most strongly associated with VTE risk [28,29], did not increase during AAS use. Nevertheless, D-dimer increased and CLT was prolonged, which suggests enhanced activation of

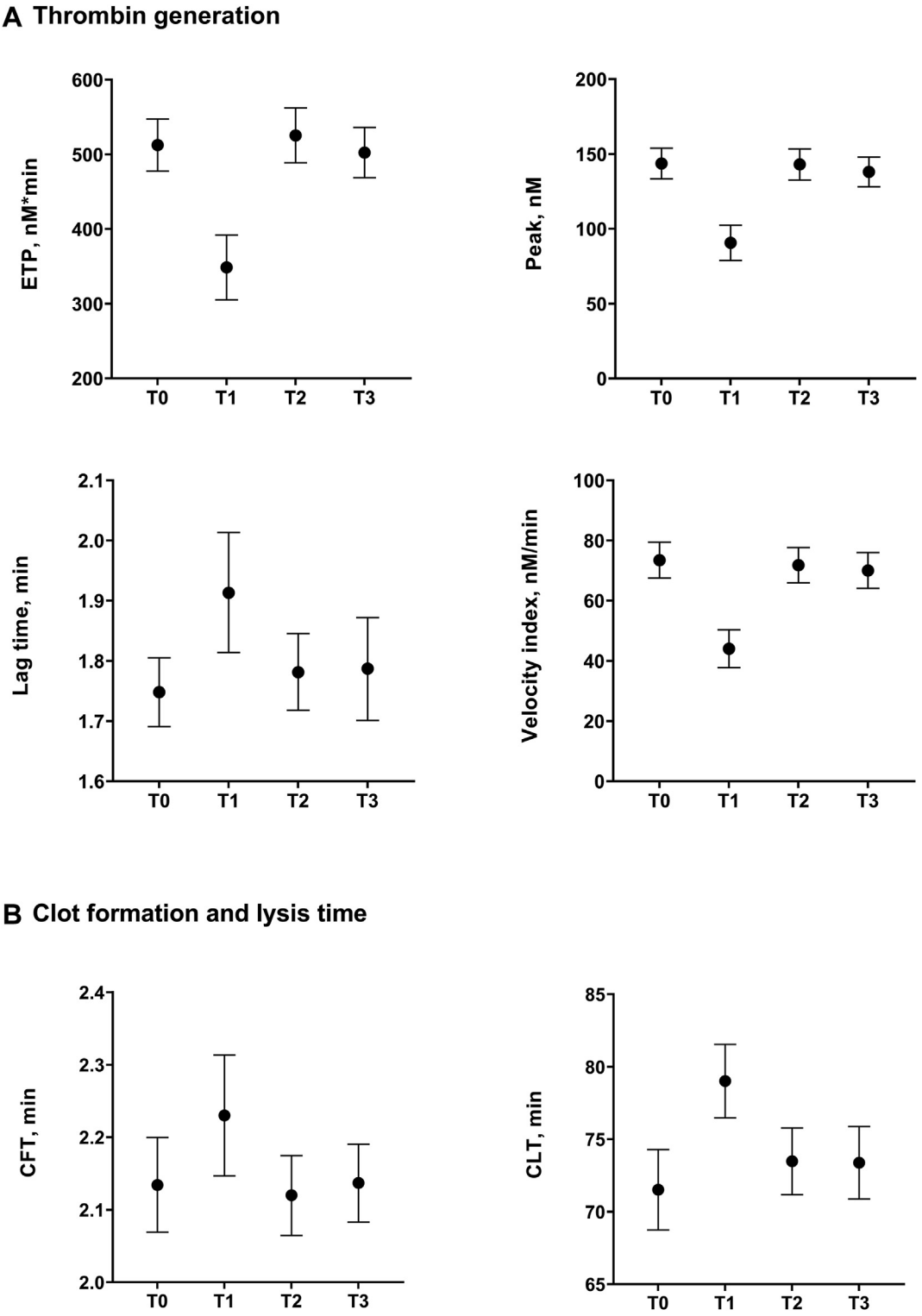

FIGURE 2 Estimated marginal means of thrombin generation parameters (A) and clot formation and lysis time (B) at baseline, during and after AAS use, obtained from a linear mixed model. Anchored lines indicate 95% CIs. CFT, clot formation time; CLT, clot lysis time; ETP, endogenous thrombin potential.

coagulation *in vivo* and decreased fibrinolytic potential. Therefore, it is difficult to draw implications on VTE risk. Studies that assess the absolute incidence of VTE during AAS use remain the benchmark to assess the real risk. Unfortunately, no such study has been performed to date. A retrospective cohort study reported a 5-fold increased risk of VTE in abusers of AAS compared with matched controls, but selection bias was an important issue [30]. A recent meta-analysis in patients using testosterone replacement therapy did not show an

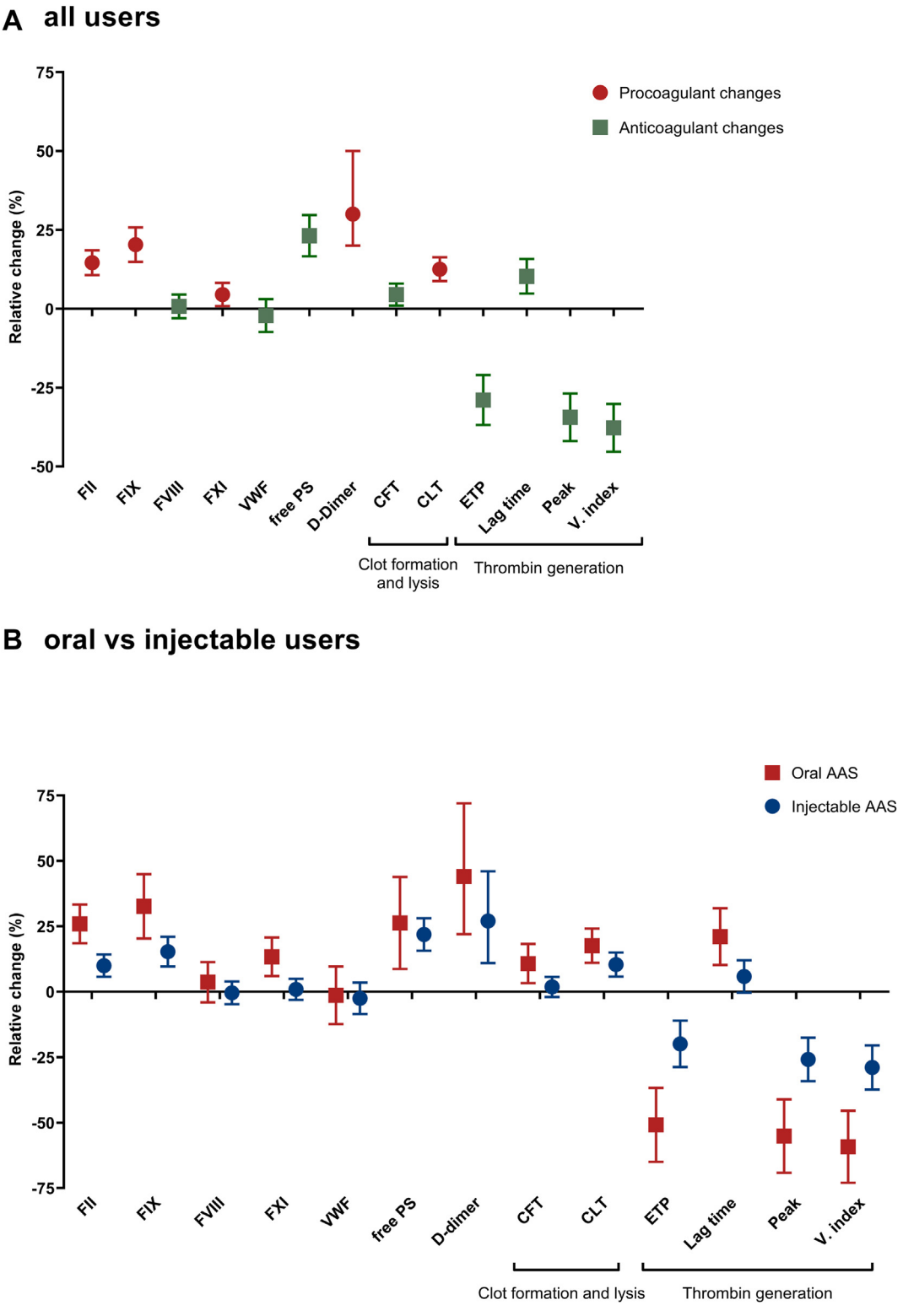

FIGURE 3 Percentage change in the coagulation parameters between baseline (T_0) and during AAS use (T_1), for all users and stratified by route of administration of AAS (oral vs injectable only). In panel A, the red circle indicates a procoagulant direction of the effect of the change in measurement, whereas a green square indicates an anticoagulant direction of the effect of the change in measurement. In panel B, the red square indicates oral users and the blue circle injectable users. Anchored lines indicate 95% CIs. CFT, clot formation time; CLT, clot lysis time; ETP, endogenous thrombin potential; F, factor; PS, protein S; V.index, velocity index; VWF, von Willebrand factor.

TABLE 2 Association between dose and cycle duration of AASs and changes in coagulation parameters between the last week (T_1) and the start (T_0) of the cycle.

	Mean ΔT_1 increase by 100-mg increase of AAS weekly dose		Mean ΔT_1 increase by 1-wk increase of cycle length	
	Mean ΔT_1 increase (95% CI)	Adjusted mean ΔT_1 increase[a] (95% CI)	Mean ΔT_1 increase (95% CI)	Adjusted mean ΔT_1 increase[a] (95% CI)
ΔFII (%)	0.7 (0.1 to 1.4)	−0.03 (−0.7 to 0.7)	−0.1 (−0.4 to 0.3)	−0.4 (−0.8 to −0.03)
ΔFVIII (%)	0.3 (−0.5 to 1.1)	−0.1 (−1.0 to 0.8)	−0.1 (−0.5 to 0.3)	−0.1 (−0.6 to 0.3)
ΔFIX (%)	1.0 (0 to 2.0)	0.2 (−0.9 to 1.3)	−0.3 (−0.9 to 0.2)	−0.5 (−1.0 to 0.1)
ΔFXI (%)	0.4 (−0.4 to 1.1)	−0.3 (−1.1 to 0.5)	−0.3 (−0.7 to 0.1)	−0.5 (−0.9 to −0.1)
ΔVWF (%)	0.3 (−1.0 to 1.6)	−0.1 (−1.6 to 1.4)	−0.3 (−1.0 to 0.4)	−0.3 (−1.1 to 0.5)
ΔPS (%)	2.1 (0.9 to 3.3)	2.3 (1.0 to 3.7)	−0.4 (−1.0 to 0.3)	−0.3 (−1.0 to 0.5)
ΔD-dimer (ng/mL)	−5.6 (−24.8 to 13.5)	−17.1 (−39.0 to 5.5)	−3.6 (−13.7 to 6.5)	−6.0 (−17.5 to 5.5)
ΔCFT (min)	0.01 (−0.002 to 0.03)	0.003 (−0.01 to 0.02)	−0.005 (−0.01 to 0.003)	−0.007 (−0.02 to 0.001)
ΔCLT (min)	0.4 (−0.2 to 0.9)	0.1 (−0.5 to 0.7)	−0.3 (−0.6 to 0.0003)	−0.3 (−0.7 to 0.04)
ΔETP (nM*min)	−1.4 (−9.2 to 6.3)	7.7 (−0.4 to 15.8)	0.7 (−3.3 to 4.7)	5.0 (0.8 to 9.3)
ΔLagtime (min)	0.02 (−0.001 to 0.03)	0.01 (−0.01 to 0.03)	−0.006 (−0.01 to 0.003)	−0.006 (−0.02 to 0.004)
ΔPeak (nM)	−0.2 (−2.3 to 1.9)	2.1 (−0.2 to 4.3)	0.2 (−0.9 to 1.3)	1.2 (−0.02 to 2.4)
ΔV. index (nM/min)	−0.02 (−1.1 to 1.1)	1.0 (−0.1 to 2.3)	0.1 (−0.4 to 0.7)	0.5 (−0.1 to 1.1)

ΔT_1 = the difference in coagulation parameters between T_1 (in the last week of the cycle) and T_0 (before the start of the cycle) from the linear regression model.

[a]Adjusted for number of different agents used, the use of AAS at time of T_1, the use of other performance and image-enhancing drugs (eg, growth hormone), medication (selective estrogen receptor modulators and antiestrogenic, aromatase inhibitors), recreational drug use.

increased risk of VTE [31], but the wide confidence intervals cannot completely rule it out.

Our study has several important strengths. We were able to prospectively investigate a relatively large group of AAS users, the largest to date, and to follow them for a total of 2 years. Previous studies on AAS users were only cross-sectional, which weakens any possible causal interpretation of the findings, and only included a limited number of subjects [11,14,23]. The use of thrombomodulin-modified TGA allowed us to include the anticoagulant protein C pathway and to obtain a complete picture of the overall hemostatic balance. Moreover, the use of the single-subject design, in which each subject acts as their own control, reduced problems with incomparability of groups (minimizing confounding from fixed factors such as age and genetics) and with sampling bias that can be introduced by selection of controls.

Other aspects should be mentioned as possible limitations. Since the study design urged subjects to completely discontinue AAS after their cycle, it excluded users who intended to use AAS continuously (ie, not in a cycle). Moreover, the study provided subjects the opportunity to monitor health for free, even though the results were disclosed only after completing the study. This may have led to, on the one hand, a cohort composed of more health-conscious subjects who use AAS more moderately, or, on the other hand, users who did not feel the need to hold back as their health was checked anyway. In addition, the AAS were not provided by the investigators and were acquired by the participants. Therefore, we cannot be sure what type and actual dose of AAS were used. A qualitative analysis performed in a random 50% of the population showed that only half of the samples contained the AAS as declared on the label [16]. Due to practical and financial limitations, we were unable to test and quantify the AAS used by all subjects and used the label information for our study. However, the use of label information to calculate the cycle dose in the analysis probably led to an underestimation of the dosage effect as it did not reflect true AAS dose. Furthermore, our results reflect the effect of stacking of AAS compounds in the uncontrolled real-life setting. Regarding loss to follow-up, only 3% of the initially planned 400 clinic visits were missed, mostly due to work obligation or personal circumstances, and never for health-related reasons. Therefore, reasons for absence until T_3 were mostly random. Moreover, enhanced training due to AAS use might partially explain the coagulation changes, as participants trained a median of 70 minutes more per week in the gym during steroid. As the subjects were using other PIEDs and medication during the cycle, as well as recreational drugs, confounding by the use of other substances cannot be excluded. Nevertheless, the similar results of the analysis adjusted for use of other PIEDs and recreational drugs, as well as of our sensitivity analysis excluding users of drugs of abuse, strengthen our results. Information on race or ethnicity of the participants was not recorded, which will not affect the validity of our results (as within-individual comparisons were made) but might affect their generalizability.

Finally, as we only measured a selection of procoagulant and anticoagulant parameters, we were not able to study the underlying mechanism of the changes in the greatest detail. For example, we only measured VWF antigen levels and did not assess potential changes in VWF activity. It is possible that low-level timely release of high-molecular-weight VWF from the endothelium could increase overall VWF activity without affecting VWF levels. Furthermore, additional measurements of other factors, such as activated protein C, TFPI, or plasminogen activator inhibitor-1, might help in clarifying the overall shift in the balance. However, our main aim was to exclude a clear procoagulant state, and therefore, we opted to include the parameters that are known to be mostly related with VTE risk.

In conclusion, we observed changes in procoagulant and anticoagulant parameters in a population of amateur strength athletes during androgen exposure. The observed changes do not point toward a clear procoagulant state during steroid use, even though the increase in D-dimer level suggests enhanced activation of coagulation and fibrinolysis *in vivo*. Therefore, further studies on AAS amateur users, powered to investigate an increased incidence of VTE, are necessary.

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AUTHOR CONTRIBUTIONS

E.C., D.L.S., N.v.R., S.C.C., and W.d.R. designed the research. D.L.S. and W.d.R. collected the data. E.C. and N.v.R analyzed the data. E.C. and N.v.R. wrote the manuscript. E.C., D.L.S., N.v.R., S.L.C., O.d.H., M.d.H., T.L., S.C.C., and W.d.R. revised the paper for important intellectual content.

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RELATIONSHIP DISCLOSURE

There are no competing interests to disclose.

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SUPPLEMENTARY MATERIAL

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